

WHAT IS CLAIMED IS:

1. A method for normalizing and amplifying an RNA population comprising the steps of:  
copying message RNA (mRNA) to form first single-stranded (ss) cDNA;  
converting the first ss-cDNA to first double-stranded (ds) cDNA;  
linearly amplifying the first ds-cDNA to form first amplified RNA (aRNA);  
5 tagging the 3' end of the first aRNA with a known sequence to form 3'-tagged first  
aRNA;  
copying the 3'-tagged first aRNA to form second ss-cDNA; and  
normalizing the mRNA or the first aRNA.

10 2. A method according to claim 1, wherein the normalizing step comprises normalizing the  
mRNA.

15 3. A method according to claim 1, wherein the normalizing step comprises normalizing the  
first aRNA.

20 4. A method according to claim 1, wherein the normalizing step comprises normalizing both  
the mRNA and the first aRNA.

25 5. A method according to claim 1, further comprising the steps of:  
converting the second ss-cDNA to second ds-cDNA;  
linearly amplifying the second ds-cDNA to form second aRNA.

30 6. A method according to claim 1, further comprising the steps of:  
converting the second ss-cDNA to second ds-cDNA;  
linearly amplifying the second ds-cDNA to form second aRNA; and  
normalizing the second aRNA.

7. A method according to claim 1, further comprising the steps of:  
converting the second ss-cDNA to second ds-cDNA;  
linearly amplifying the second ds-cDNA to form second aRNA;

normalizing the second aRNA;  
copying the second aRNA to form third ss-cDNA;  
converting the third ss-cDNA to third ds-cDNA; and  
linearly amplifying the third ds-cDNA to form third aRNA.

5 8. A method according to claim 1, wherein the tagging step comprises contacting the first aRNA with an oligonucleotide and a ligase, whereby the ligase adds the oligonucleotide to the 3' end of the first aRNA to form the 3'-tagged first aRNA.

10 9. A method according to claim 1, wherein the tagging step comprises contacting the first aRNA with a nucleotide and a polyadenyltransferase under conditions whereby the polyadenyltransferase adds the nucleotide to the 3' end of the first aRNA to form the 3' tagged first aRNA.

15 10. A method according to claim 1, wherein the converting step comprises:  
contacting the first ss-cDNA with RNase H and a DNA polymerase under conditions whereby the RNase H nicks the associated mRNA and the DNA polymerase initiates conversion at a noncovalently joined heteroduplex region and copies the first ss-cDNA to the first ds-cDNA.

20 11. A method according to claim 1, wherein the converting step comprises:  
contacting the first ss-cDNA with RNase H and a DNA polymerase under conditions whereby the RNase H nicks the associated mRNA and the DNA polymerase initiates conversion at a noncovalently joined heteroduplex region and copies the first ss-cDNA to the first ds-cDNA, wherein the polymerase provides 5' exonuclease activity.

25 12. A method according to claim 1, wherein the converting step comprises:  
contacting the first ss-cDNA with RNase H and a DNA polymerase under conditions whereby the RNase H nicks the associated mRNA and the DNA polymerase initiates conversion at a noncovalently joined heteroduplex region and copies the first ss-cDNA to the  
30 first ds-cDNA, wherein the polymerase lacks 5' exonuclease activity.

13. A method according to claim 1, wherein the mRNA copying step comprises:  
contacting the mRNA with a primer comprising an oligo dT sequence, an RNA  
polymerase promoter and an affinity tag.

14. A method according to claim 1, wherein the mRNA copying step comprises:  
contacting the mRNA with a primer comprising an oligo dT sequence, an RNA  
polymerase promoter and an affinity tag, wherein the affinity tag is biotin.

15. A method according to claim 1, wherein the mRNA copying step comprises:  
contacting the mRNA with a primer comprising an oligo dT sequence, an RNA  
polymerase promoter and an affinity tag, wherein the promoter activates an RNA polymerase  
selected from the group consisting of T7, T3 and SP6.

16. A method according to claim 1, wherein the amplifying step is effected with an RNA  
polymerase selected from the group consisting of T7, T3 and SP6.

17. A method according to claim 1, wherein the normalizing step comprises hybridizing  
the mRNA or the first aRNA with driver polynucleotides and then separating an unhybridized  
fraction of the mRNA or first aRNA.

18. A method according to claim 1, wherein the normalizing step comprises hybridizing  
the mRNA or the first aRNA with driver polynucleotides and then separating an unhybridized  
fraction of the mRNA or first aRNA, wherein the separating step is effected by a method  
selected from the group consisting of hydroxyapatite-based affinity separation and biotin-  
streptavidin-based affinity separation.

19. A kit for normalizing and amplifying an RNA population, said kit comprising  
instructions describing the method of claim 1 and a premeasured portion of a reagent selected  
from the group consisting of: oligo dT T7 biotinylated primer, T7 RNA polymerase, annealed  
biotinylated primers, streptavidin beads, polyadenyl transferase, reverse transcriptase, RNase  
H, DNA pol I, buffers and nucleotides.

20. A kit according to claim 20, comprising premeasured portions of oligo dT T7 biotinylated primer, T7 RNA polymerase, annealed biotinylated primers, streptavidin beads, polyadenyl transferase, reverse transcriptase, RNase H, DNA pol I, buffers and nucleotides.

21. A method for making tagged driver RNA for normalizing and amplifying an RNA population comprising the steps of:

(a) combining linkers with a population of RNA species,

wherein each linker comprises a first oligo comprising a first tagged annealing region and a second oligo comprising in 5' - 3' direction a second tagged annealing region and a capture region, wherein the annealing regions are complementary and annealed to each other; and

each RNA species comprises a target region complementary to the capture region, under conditions wherein the target regions hybridize to the capture regions; and

(b) ligating the target regions to the first tagged annealing region to form tagged driver RNA.

22. A method according to claim 21, wherein the tagged annealing regions comprises a tag and the tag is biotin.

23. A method according to claim 21, wherein the population is of mRNA species, the capture region is polydT and the target region is polyA.